Controlled Human Exposure to Methyl Tertiary Butyl Ether in Gasoline: Symptoms, Psychophysiologic and Neurobehavioral Responses of Self-Reported Sensitive Persons

Nancy Fiedler,[1,2] Kathie Kelly-McNeil,[1,2] Sandra Mohr,[1,2] Paul Lehrer,[1] Richard E. Opiekun,[1,2] ChiaWei, Lee,[1,2] Tom Wainman,[1,2] Robert Hamer,[1] Clifford Weisel,[1,2] Robert Edelberg,[1,2] and Paul J. Lioy[1,2]

[1]UMDNJ-Robert Wood Johnson Medical School, Department of Environmental and Community Medicine, Piscataway, New Jersey, USA; [2]Environmental and Occupational Health Sciences Institute, Piscataway, New Jersey, USA.

The 1990 Clean Air Act mandated oxygenation of gasoline in regions where carbon monoxide standards were not met. To achieve this standard, methyl tertiary butyl ether (MTBE) was increased to 15% by volume during winter months in many locations. Subsequent to the increase of MTBE in gasoline, commuters reported increases in symptoms such as headache, nausea, and eye, nose, and throat irritation. The present study compared 12 individuals selected based on self-report of symptoms (self-reported sensitives; SRSs) associated with MTBE to 19 controls without self-reported sensitivities. In a double-blind, repeated measures, controlled exposure, subjects were exposed for 15 min to clean air, gasoline, gasoline with 11% MTBE, and gasoline with 15% MTBE. Symptoms, odor ratings, neurobehavioral performance on a task of driving simulation, and psychophysiologic responses (heart and respiration rate, end-tidal CO_2, finger pulse volume, electromyograph, finger temperature) were measured before, during, and immediately after exposure. Relative to controls, SRSs reported significantly more total symptoms when exposed to gasoline with 15% MTBE than when exposed to gasoline with 11% MTBE or to clean air. However, these differences in symptoms were not accompanied by significant differences in neurobehavioral performance or psychophysiologic responses. No significant differences in symptoms or neurobehavioral or psychophysiologic responses were observed when exposure to gasoline with 11% MTBE was compared to clean air or to gasoline. Thus, the present study, although showing increased total symptoms among SRSs when exposed to gasoline with 15% MTBE, did not support a dose–response relationship for MTBE exposure nor the symptom specificity associated with MTBE in epidemiologic studies. *Key words*: controlled exposure, methyl tertiary butyl ether, neurologic, psychophysiology, self-reported sensitives, symptoms. *Environ Health Perspect* 108:753–763 (2000). [Online 28 June 2000]
http://ehpnet1.niehs.nih.gov/docs/2000/108p753-763fiedler/abstract.html

Until the fall of 1992, methyl tertiary butyl ether (MTBE) was used as an octane enhancer in premium gasolines. To bring several regions of the United States into compliance with the National Ambient Air Quality Standards for carbon monoxide, the 1990 Clean Air Act (*1*) mandated 2.7% by weight oxygen concentration in cold season fuels and 2.0% by weight oxygen during photochemical smog season. Although both MTBE and ethanol can be used to oxygenate fuel, MTBE was the chemical most frequently chosen for oxygenation.

This increase to 15% MTBE in gasoline was followed by acute health complaints in several regions of New Jersey and Alaska (*2,3*). Subsequent cross-sectional community studies of healthy workers suggested that the highest blood levels of MTBE were associated with more symptoms (*4–6*). Controlled exposures to pure MTBE, however, did not show increased symptom reports or neurobehavioral performance deficits in healthy young adults (*7–9*). To date, no controlled exposure to MTBE has directly evaluated the symptoms and health effects that were reported anecdotally by a subset of individuals (*7,10*). Therefore, the purpose of the present controlled exposure study was to compare the symptoms, psychophysiologic reactivity, and neurobehavioral performance of persons who reported sensitivity to MTBE and controls in response to four controlled exposure conditions: clean air, gasoline, gasoline with 11% MTBE, and gasoline with 15% MTBE.

Three controlled exposure studies have investigated the effect of pure MTBE on symptoms and objective measures of irritation and performance in healthy subjects. Measures of eye and nasal inflammation were chosen as physiologic measures of reported irritant symptoms; neurobehavioral measures were performance-based indicators of symptoms, such as disorientation, presumed to interfere with tasks that rely on speed of response (e.g., driving).

Using a double-blind cross-over design, Prah et al. (*8*) exposed healthy subjects to 1.4 ppm MTBE versus clean air for 1 hr. Cain et al. (*9*) exposed healthy subjects for 1 hr to each of three conditions: 1.7 ppm MTBE, 7.1 ppm mixture of 17 volatile organic compounds, and clean air. Neither study found an increase in symptoms, a reduction in neurobehavioral performance, or changes on measures of eye irritation (tear film breakup). The number of polymorphonuclear neutrophil leukocytes in nasal lavage fluid samples was increased only 18–24 hr after exposure to volatile organic compounds (*9*). In these studies, blood levels of MTBE were higher than the upper quartile levels assessed in the cross-sectional studies of workers. More recently, Nihlen et al. (*7*) exposed 10 healthy males to 5, 25, and 50 ppm MTBE and found that subjects reported significantly higher ratings of solvent smell with higher exposure to MTBE. Ratings of odor declined over time, suggesting habituation to the odor. No increase in symptoms or changes in objective measures of eye irritation was observed (e.g., tear film breakup time, redness). Nasal airway resistance increased significantly after exposure, but the effect was not correlated with exposure. Thus, in healthy subjects, MTBE exposures under controlled conditions did not replicate the symptoms reported either anecdotally or in cross-sectional community studies. These controlled exposures to MTBE, however, were not those of typical exposures such as refueling or driving, where MTBE is encountered only as an additive to gasoline. Moreover, controlled exposure studies to date have not included self-reported sensitive individuals.

As is observed with other noxious odorants, symptomatic complaints in response to MTBE in gasoline could be secondary to emotional responses evoked by unpleasant odor (*11,12*). The association between odor, emotion, and physiologic arousal has been used to investigate psychologic and physiologic

Address correspondence to N. Fiedler, Environmental and Occupational Health Sciences Institute, UMDNJ-Robert Wood Johnson Medical School, 170 Frelinghuysen Road, Room 210, Piscataway, NJ 08854 USA. Telephone: (732) 445-0123, ext. 625. Fax: (732) 445-0130. E-mail: nfiedler@eohsi.rutgers.edu

We thank the participants who volunteered for the study, the Scientific Advisory Committee for its comments, and D. Ashley of the Centers for Disease Control for quality assurance analyses of blood data.

Funding was provided by ARCO (now Lyondell) and the New Jersey Department of Environmental Protection. N. Fiedler, C. Weisel, S. Mohr, and P. Lioy are supported, in part, by NIEHS Environmental Health Center grant ES05022-12.

Received 31 January 2000; accepted 11 April 2000.

responses to various pleasant and unpleasant odors (*13,14*). For example, subjects with greater fear of undergoing dental work responded with increased autonomic arousal (i.e., electrodermal changes) to eugenol, an odorant associated with dental procedures (*15*). The hypothesis of a relationship between the odor of MTBE and a symptomatic response to MTBE is further substantiated by the overlap between reported key symptoms (i.e., eye irritation, burning sensation in nose or throat, headache, nausea or vomiting, cough, sensations of "spaciness" or disorientation, dizziness) and those associated with anxiety and/or hyperventilation (e.g., spaciness, dizziness). Therefore, in addition to measuring symptoms and simulated driving performance, psychophysiologic changes associated with symptoms of anxiety such as increased heart and respiration rate, reduced blood pulse volume in the periphery, and increased end-tidal CO_2 were measured in response to exposure.

The present study evaluated symptoms, psychophysiologic responses, and neurobehavioral performance of self-reported sensitive individuals (SRSs) and controls. In addition to clean air and gasoline, subjects were exposed to 15% MTBE and 11% MTBE in gasoline at concentrations similar to those documented during refueling (*16*). This controlled exposure study was intentionally designed to test directly the anecdotal reports of self-reported sensitivity to MTBE in gasoline. Moreover, by including psychophysiologic measures, we sought to distinguish symptoms secondary to an emotional response (e.g., anxiety) evoked by the odor of the exposure from those resulting directly from exposure.

Methods

Subject Recruitment

SRS subjects from throughout New Jersey were recruited through radio and newspaper advertisements and letters sent to members of the mailing lists for the activist group Oxybusters (Plainsboro, NJ), the National Reformulated Gas Hotline of the Oxygenated Fuels Association (Arlington, VA), and the New Jersey Department of Environmental Protection (Trenton, NJ). Individuals inquiring about the study from advertisements ($n \cong 700$) and members of the mailing lists ($n = 800$) were sent an introductory letter describing the study, a general health screening questionnaire, and a survey of 34 symptoms. The symptom survey asked the individual to rate the level of discomfort associated with each symptom on a scale from 0 (do not have the symptom) to 3 (symptom is severe) and then indicate whether the discomfort occurred at a gas station or around traffic exhaust and how long the symptom lasted. For the purposes of this study, individuals who scored ≥ 6 out of 21 points (total) on MTBE-related symptoms reported to occur around gasoline (headache, cough, nausea, daytime sleepiness, burning sensation in nose or mouth, losing balance or dizziness, difficulty concentrating) (*4*) were classified as SRSs. Controls were recruited by newspaper advertisements and were sent the same screening questionnaires as for SRS recruitment. A total of 287 (19%) screening forms were returned. Twenty-five of the surveys could not be scored due to incomplete information, leaving 262 to score and categorize.

Of the questionnaires scored, 184 (70%) were classified as controls and 78 (30%) as SRSs. After classification, individuals with any of the following medical conditions were excluded from participation: neurologic disease or history of brain injury, significant exposure to neurotoxicants, stroke or cardiovascular disease, serious pulmonary disease (e.g., asthma), liver or kidney disease, serious gastrointestinal disorders, nasal polyps, nasal surgery, sinus disease, chronic fatigue syndrome, multiple chemical sensitivities, or major psychiatric disorders including psychoses, bipolar disorder, alcoholism, or drug abuse. Smokers and pregnant or lactating women were also excluded, as were individuals taking beta and alpha blockers, anxiolytics, antidepressants, and steroids.

The subjects who did not report any of the above conditions underwent a complete medical history and physical examination with a standard laboratory blood chemistry panel and electrocardiogram (EKG). Based on the results of either the screening survey or the physical examination, 53 controls and 28 SRSs were excluded from the study due to medical conditions (e.g., diabetes, hypertension, smoking) or medication use. Fifty-three controls and 38 SRSs decided not to participate for logistic reasons (e.g., moved, time constraints), and 59 controls were not invited to participate because they did not demographically match the recruited SRSs. Of the remaining 31 subjects, 19 were controls and 12 were SRSs. The number of SRSs was less than initially planned; however, no additional SRSs were either medically fit or willing to participate. Subjects were paid an incentive of $500 upon completion of all exposure sessions. If all sessions were not completed, a prorated amount was paid to the subject. Thus, the SRS subjects were those willing to participate, but also those who did not have any major or chronic physical or psychological disorder.

Subjects

Twelve SRSs (6 males, 6 females), mean age 42.3 years (SD = 11.9, range = 23–65) and 19 controls (6 males, 13 females), mean age 39.1 (SD = 9.9, range = 24–65) ($p = 0.38$) completed the protocol. SRSs had a mean of 17.1 (SD = 4.6, range = 12–18) years of education and the controls a mean of 14.9 years (SD = 2.8, range = 12–23; $p = 0.19$). In the SRS group there were 7 whites and 5 in various ethnic groups, while the controls had 16 whites and 3 of other ethnic origin ($p = 0.17$). No significant differences in age, education, sex ($p = 0.31$), or ethnicity distribution were observed between SRSs and controls. SRSs scored significantly higher than controls on MTBE-related (SRS: mean = 9.83, SD = 2.33; control: mean = 0.58, SD = 1.07; $t = -12.93$, $p < 0.0001$) and non–MTBE-related symptoms (SRS: mean = 14.08; SD = 10.54; control: mean = 0.37; SD = 1.01; $t = -4.50$, $p < 0.001$). Thus, on the screening questionnaire, SRSs reported more symptoms than controls.

Measures

Blood levels of MTBE and tertiary butyl alcohol. Pre- and postexposure blood was sampled from SRSs and controls for all experimental conditions. Samples were analyzed by both the Environmental and Occupational Health Sciences Institute and the Centers for Disease Control and Prevention (Atlanta, GA) for the presence of MTBE and its metabolite, tertiary butyl alcohol (TBA). Blood MTBE values from the two laboratories were highly and significantly correlated ($r = 0.91$), whereas TBA values were significantly but moderately correlated ($r = 0.52$).

Symptom questionnaire. A list of 42 symptoms associated with MTBE, solvent exposure, anxiety (*17*), depression (*18*), and breathing problems was used to assess symptoms during the exposure protocol (Table 1). Each symptom was rated on a scale from 0 (barely detectable/no sensation) to 100 (strongest imaginable), which was developed by Green et al. (*19*) to approximate a ratio scale. The questionnaire was completed four times during the protocol session, and symptom ratings were summed and averaged to form a total symptom score and the following symptom subscales: MTBE, anxiety, depression, breathing, solvent (Table 1). MTBE symptoms were associated with MTBE exposure from the literature on gallstone dissolution, animal toxicology, and the Alaskan studies (*20*). Although the literature offers some logical basis for use of a subset of symptoms as MTBE-related, both the Alaskan epidemiologic and gallstone studies involved other concurrent exposure (e.g., sedatives for gallstone procedures), which could contribute to the symptoms experienced. In addition to symptoms, subjects were also asked to rate the environment on a 1–5 scale for eight qualities such as lighting

intensity, noise, and temperature (*21*). A total environment score was computed by summing and averaging these ratings each time the questionnaire was given.

Odor questionnaire. The odor scale assessed odor intensity and irritation on a scale from 0 (barely detectable/no sensation) to 100 (strongest imaginable) and pleasantness (1 = very pleasant to 9 = very unpleasant) (*22*). The scales were administered at 5-min intervals during the 20-min session.

Neurobehavioral test. A computerized divided-attention test of cognitive performance, Performance On Line (POL) (*23*), was chosen because it has been shown to be sensitive to the effects of alcohol in repeated-measures studies and because it is a computerized driving simulation task that could be useful to test the effects of MTBE on cognitive functions relevant to driving. This test includes a central task in which the subject is presented with two lanes of traffic, divided by a double yellow line. Four conditions of head lights and tail lights appear on any one trial: *a*) left lane, white head lights and right lane, red tail lights; *b*) left lane, red lights and right lane, white lights; *c*) left and right lane, red lights; and *d*) left and right lane, white lights. The subject is instructed to press the space bar only when condition *a* or a safe condition exists. Additionally, a peripheral task requires that the subject respond with one of four arrow keys (up, down, left, right) in the direction of the critical stimulus (octagon shape representing a stop sign). Task difficulty increases by adding to the number of distracting stimuli in the peripheral display using a random assortment of circles, squares, and triangles of different colors. The task has five levels that progress in difficulty. The subject practices the first four levels in preparation for the fifth level, in which the subject must respond to both central and peripheral critical stimuli. After learning the task, 10 trials of 45 displays are presented at the fifth level. A composite performance score encompasses all of the responses related to the central and peripheral tasks of the test and is based on a weighted algorithm (*23*). This score accounts for the speed and accuracy of all responses and was the only score used for analysis of performance.

Table 1. Symptom questionnaire subscales.

Symptom	Manifestation
MTBE[a]	Throat irritation
	Eye irritation
	Coughing
	Nose irritation, dryness, or itching
	Tired or sleepy
	Headache
	Nausea
	Dizzy
	Disoriented
Breathing[a]	Panting
	Wheezy
	Chest tightening
	Choking
Anxiety[a]	Jittery
	Short of breath
	Nervous
Depression[a]	Everything is an effort
	Feel sad
	Depressed
	Heart beating faster
	Worried
	Tense
Solvent[a]	Runny nose
	Lightheaded
	Bad taste
	Sneeze
	Nasal congestion
	Stomachache
	Heart palpitations
	Ear ringing
	Itching
	Leg cramps
	Skin irritation or dryness
	Perspiring
	Leg/arm tickling, tingling, numb
	Drowsy
	Difficulty concentrating
	Chest pain
	Fatigued
	Back pain
	Muscle or joint pain
	Runny eyes
Environment[b]	Lighting intensity
	Noise level
	Room temperature
	Humidity
	Air movement
	Air quality
	Odor level
	Ventilate room

[a]Rating scale: 0 (barely detectable/no sensation) to 100 (strongest imaginable). [b]Rating scale: 1 (low) to 5 (high).

Psychophysiologic measures. We used the Flexcomp Biomonitoring System 1.51B (Thought Technology Limited, Montreal, Quebec, Canada) to collect heart and respiration rate, surface electromyograph (EMG) from the frontalis area, finger pulse volume, and finger temperature as indicators of physiologic responses to the exposure conditions. Cardiac data (16 samples/sec) were collected using disposable EKG electrodes with one electrode placed over the right mid-clavicular area, one over the left mid-clavicular area and the third placed just below the sternal notch as the ground lead. EMG (16 samples/sec) was obtained from disposable electrodes with the positive electrode positioned approximately 2 cm over the right eye, the negative 2 cm over the left eye, and the ground electrode in the center of the forehead. This placement detects activity throughout the facial area and is significantly affected by emotional changes (*24*). Finger temperature (1 sample/sec) was assessed using a thermister and Flexcomp software, and finger pulse volume (3 samples/sec) via an LED photoplethysmograph and peak detection software developed in our laboratory. The Criticare Systems Inc. Pulse Oximeter End Tidal (POET) II (Waukesha, WI) CO_2 monitor was used to collect end-tidal CO_2 (31 samples/sec) and respiration count through a nasal catheter and cannula. End-tidal CO_2 is a noninvasive measure that is closely related to $PaCO_2$ (*25,26*).

Delivery system. Approximately 70 mL of the gasoline mixture was poured into a 100-mL three-necked Pyrex flask. The two unused necks were capped tightly to prevent evaporation of the gasoline mixture. A length of Teflon tubing was inserted into the center neck of the flask and connected to an adjustable stroke laboratory pump (model V100; Fluid Metering, Inc., Oyster Bay, NY). The pump consisted of a piston (model H00) made from stainless steel encased in a plastic cylinder and lined with a sintered carbon liner. After passing through the pump, the tubing containing the gasoline mixture passed through a pulse dampener to suppress the flow pulse. Once the pulse had been suppressed, the gasoline mixture passed through a capillary tubing to a Y-connecter on the top of a condenser column seated atop a 500-mL three-necked flask placed within a 45°C heated water bath (#66648; Precision Scientific, Chicago, IL). A fiberglass wick was placed at the end of the capillary tubing and was used to control dripping and delivery of gasoline into the column. As the wick became saturated, a small drop of gasoline would drip through the column, where a heated air stream (4 L/min) would strip the volatile components of the mixture from the drop and deliver the vapor back up through the column to the top of a Y-connector on the condenser column, which was connected to heated tubing at 243°C. The tubing (model # LP212-4-50; Technical Heaters, Inc, San Fernando, CA), with low-pressure Teflon core and tin/copper overbraid, was connected into the air delivery system of the controlled environment facility (CEF) at the diffusers. The system was designed to achieve a 15% MTBE level of 1.7 ppm in the CEF. Further, the delivery system would not vaporize the entire gasoline droplet because such a scenario would deliver compounds to the subject not normally inhaled by a commuter.

The water bath contained a 1/125 horsepower submersible circulating pump (model 4182K22; McMaster-Carr, New Brunswick, NJ), operating at 2.8 gal/min, connected to a reflux condenser and a 500-mL three-necked flask, which collected the heavier components of the gasoline. The flask contained a thermometer to ensure the temperature was constant. An air line connected to a small air pump (MEDO, Hanover Park, IL) was placed into the neck of the flask to strip off the more volatile constituents of the gasoline

mixture and deliver vapors through the heated tubing at 4 L/min. The circulating pump was used to draw water from the heated water bath and deliver it through the column. The exposed glass Y-connector at the top of the column was covered with glass wool and wrapped with a heated tape (McMaster-Carr) to ensure that the system was at a constant temperature and that the vapors did not cool before entering the heated tubing.

Gasoline mixture storage and handling. Winter blend gasoline (i.e., with no oxygenates) (Sunoco Custom Blends, Philadelphia, PA) was stored in a tightly sealed 55-gal drum in an enclosed external storage facility. It was siphoned into a clean 3.9-L Qorpak amber safety-coated container (Fisher Scientific, Pittsburgh, PA) as needed using a Teflon-lined manual siphon. The same gasoline was used for the nonoxygenated gasoline exposure samples and to make the two blends of oxygenated fuels: gasoline without oxygenate was mixed with 15% (v/v) MTBE (1.7 ppm in air) (Mallinckrodt Baker, Paris, KY) to simulate the winter blend of gasoline, and with 11% (v/v) MTBE (1.1 ppm in air) to simulate the concentration of MTBE added to a reformulated gasoline. Both blends were stored in separate, tightly sealed glass jars and kept in a cold room at 4°C. Before use, a blend was allowed to equilibrate to room temperature.

Vapor monitoring. A gas chromatograph, with a DB-VRX column (J&W Scientific, Folsom, California) initially heated to 35°C, was used to determine the MTBE concentration within the facility approximately 8 min into the exposure session. The temperature of the column was held for 10 min and increased at a rate of 5°C/min to a final temperature of 150°C, where it was again held for 10 min. The injector and detector were maintained at 200°C. The MTBE samples were collected in sorbant traps made of stainless steel tubes (0.5 cm id × 8.8 cm; Perkin-Elmer, Inc., Norwalk, CT) and were packed with Carboxen 569 (Supelco Inc., Bellefonte, Pa). The trap was filled with 0.6 g Carboxen 569 and held in place by glass wool and gauze on both ends. Each trap was conditioned in an oven for 6 hr at 340°C. All conditioned traps were sealed with a Teflon cap and stored in a desiccator before use. The absorbed MTBE was transferred to a capillary gas chromatograph (5890; Hewlett-Packard) with a DB-5 capillary column (J&W Scientific) for analysis using an automated thermal desorption system (model ATD-400; Perkin-Elmer, Inc.). Quantification was completed using a Hewlett-Packard 5970 mass selective detector. The total hydrocarbon concentration in the CEF, evaporated from gasoline, was 33 ± 0.5 ppm, measured and reported as methane by a total hydrocarbon analyzer (model 23-500; GOW-MAC, Lehigh Valley, PA). The sample pressure setting on the total hydrocarbon analyzer was maintained at 2.5 psi, and the air and fuel pressure settings were maintained at 25 psi and 7.0 psi, respectively. There was a consistent relationship between the total hydrocarbon level and the level of MTBE present in gasoline. Thus, the total hydrocarbon levels were used as a continuous indicator of MTBE levels. This result was verified by analyzing air in the CEF for MTBE by gas chromatography in a grab sample. A final verification of the MTBE level during an individual exposure was the analysis of a sample of the average level of MTBE over the entire session. For gasoline with 15% MTBE, the average CEF concentration was 1.7 ppm, and for gasoline with 11% MTBE, the average CEF concentration was 1.1 ppm.

Controlled environment facility. The facility is a 7.3 ft (height) by 13.5 ft (width) by 9 ft (depth) stainless-steel room in which air flow, temperature, and humidity are varied and controlled. The facility was operated at 70 ± 1°F during each exposure session. The relative humidity range was maintained at 45 ± 2% for each exposure session. Air flow was maintained between 650 and 700 cubic feet per minute (cfm), and the facility was operated under negative pressure of 0.1 inches of water. The nonrecirculated air supply passed through a sequence of conditioning processes, which included air cooling and heating, humidification and dehumidification, and filtration through 12 carbon filters and 1 HEPA filter. The air supply entered the facility through two diffusers in the ceiling and exited through the perforated stainless-steel floor to exhaust vents. Air concentrations for exposures were maintained by constant injection of compounds into the air supply, which flows through the room without recirculation. The gasoline mixture was introduced into the air supply by means of an evaporation delivery system and was well-mixed before entering the facility to achieve a uniform concentration throughout its volume. The desired concentration within the CEF was attained within 5 min. To ensure that a uniform concentration was maintained after delivery of the mixture into the chamber, a 12-inch, three-speed oscillating fan was kept running on low speed during an exposure session. Levels of total hydrocarbons were continuously monitored remotely to ensure that experimental levels were maintained for the duration of the session. Study participants entered the facility through an air lock which had two doors; one opened to the outer room and the other into the facility. Throughout the experimental exposure, subjects were observed through a two-way window and contact was maintained via an intercom.

Procedure

For the orientation visit, subjects reported to the clinical center, where they gave informed consent in accordance with standard Institutional Review Board procedures. A complete medical history, a physical examination, and an EKG were performed on each subject. To reduce anticipatory anxiety and practice effects on the neurobehavioral task, subjects learned to use the POL driving simulation software by performing levels 1–5. Subjects then completed an unblind baseline session in the CEF to reduce initial anxiety and to familiarize them with the procedures and surroundings. All sessions including the unblind orientation session occurred in the morning to control for circadian rhythm variation and followed the same procedures as outlined below.

Exposure sessions were double blind. Previous research to test the ability of SRSs and controls to discriminate gasoline alone from gasoline with 15% MTBE and from gasoline with 15% MTBE and a re-odorant found that subjects from both groups performed no better than chance. Subjects were asked to identify two mixtures as "same" or "different" when the mixtures were presented in pairs of standard sniff bottles (*27*). Therefore, based on this inability to distinguish gasoline from gasoline with 15% MTBE, no re-odorant was added to the exposure conditions to "mask" the odor of MTBE. Moreover, we assumed that exposure conditions were blind to subjects, and this assumption was also tested by asking subjects to guess the exposure condition.

SRS subjects were randomly assigned to an order of exposure, and their demographically matched controls were assigned to the same order. For each exposure visit, subjects were admitted by a nurse who ascertained that they did not have a viral illness, were not pregnant, and had not taken medications that would disallow participation. Subjects had EKG electrodes attached, completed 10 trials of POL at level 5 as baseline neurobehavioral performance, and were escorted to the CEF. The subject was then instructed about safety procedures and location of emergency exits, given a Tyvek suit to protect clothing, and electrodes for frontalis EMG recording, finger pulse volume, and finger temperature were attached by the experimenter. A sterile, non-reusable cannula for end-tidal CO_2 recording was inserted approximately 1/4 inch inside the nose and held in place with surgical adhesive tape. The subject was then seated in a comfortable, nonreclining chair with the computer keyboard and monitor in front of him or her on a stainless-steel table, and the experimenter left to begin the session. The experimenter constantly observed the subject through a one-way

window, and an intercom was used for communication. The subject completed the first symptom questionnaire and was then asked to sit quietly, relax, breathe through the nose, and keep eyes open for the next 20 min. Throughout this period, the subject viewed the computer monitor, where a vigilance task randomly displayed bars on the screen and a tone sounded occasionally to maintain the subject's attention. The subject was instructed to keep a mental count of the bars and then report the total number when asked at the end of the experimental session. Additionally, during this 20-min period, the subject heard a warbling sound every 5 min after which an odor survey was completed.

All sessions began with a 5-min relaxation period, during which baseline psychophysiologic measures were taken. For those sessions in which exposure to gasoline with or without MTBE occurred, exposure began after the 5-min relaxation or baseline period and lasted for 15 min (Figure 1). The subject was not informed when the exposure began. Therefore, the only exposure cue was perceived odor. During the 15-min exposure period, the subject sat quietly and counted the bars on the monitor. Immediately after exposure, psychophysiologic measurements were discontinued, and the subject was instructed to remove all the monitoring leads to which he or she was connected. The subject was instructed to complete another symptom questionnaire and then performed 10 trials of POL at level 5. Before leaving the CEF, the subject was asked to guess the exposure condition from four possible choices (no exposure; gasoline with no MTBE, gasoline with low MTBE, gasoline with high MTBE), removed the Tyvek suit, and was escorted to the clinical center. The subject completed a postexposure symptom questionnaire, performed 10 trials of POL at level 5, and filled in another symptom questionnaire just before leaving for the day. Each visit lasted approximately 3 hr.

Hypotheses and Statistical Analysis

Symptoms, neurobehavioral performance, and psychophysiologic variables were analyzed as separate variable groups. Because data for each of these variables sets were frequently not normally distributed, permutation tests were used to assess group differences. For example, approximately 50% of the ratings for symptom variables were zero.

Permutation (or randomization) tests fall into the same general class of statistical methods as bootstrapping, resampling techniques (*28*). Permutation tests enumerate all possible permutations of the data permissible under the null hypothesis, and examine the observed sample test statistic in the context of this permutation distribution. They do not assume normality. For example, suppose we had two samples of size 5 each, and we measured each observation on some numeric response variable; if we could assume that in the populations from which these samples were drawn that the variable is normally distributed, then an independent-groups t-test would be an appropriate statistical test. If we cannot assume normality, then a t-test is inappropriate with this sample size. (A nonparametric test is a common alternative, but is less powerful than the parametric test.) Under the null hypothesis of no group mean difference, it is equally likely that a particular score would occur in one group as in the other group. That is, under the null hypothesis, there really is one population. The division of the observations into two groups is simply random if the null hypothesis is true. The null hypothesis is examined by determining how unusual the mean difference observed actually is by generating all possible permutations of the data: all possible ways of dividing these 10 observations into two groups of 5 each (i.e., 252 possible divisions). For each of these 252 permutations, the observed value of the test statistic is calculated (t-statistic). The p-value or the probability of observing a test statistic as extreme or more extreme under the null hypothesis is calculated. For example, suppose that the t observed was the largest t-statistic in the distribution of 252 t-statistics generated from permutations of the data; the probability of observing a t-statistic as extreme or more extreme than the one observed would be 1/252, or 0.004. Because this p-value is less than the predetermined significance level of 0.05, the null hypothesis is rejected.

When total scores for symptoms and neurobehavioral performance were used, corrections for multiple comparisons were not made. However, if the overall total score was significant, we analyzed subscale scores and applied the Bonferroni correction for multiple comparisons. For odor ratings, a mixed model, with group as a between-subjects factor and exposure and time as within-subjects factors, was used. Lack of sphericity was corrected for with a Hyunh-Feldt correction.

The hypothesis for symptom data was that relative to controls, SRSs would report more symptoms in each of the exposure conditions (i.e., gasoline, gasoline with 11% MTBE, gasoline with 15% MTBE). To test this hypothesis, baseline symptom scores (1–5 min) were subtracted from postexposure symptom scores for each subject; a difference score between exposure conditions of the postexposure/baseline difference scores was computed as follows using gas with 15% MTBE versus clean air as an example:

$$\frac{\sum_{1\text{–}N}\left(\text{Post exposure G15}_{(1\text{–}N)} - \text{Baseline G15}_{(1\text{–}N)}\right)}{N} - \frac{\sum_{1\text{–}N}\left(\text{Post exposure CA}_{(1\text{–}N)} - \text{Baseline CA}_{(1\text{–}N)}\right)}{N}$$

These differences were then compared between groups (SRSs/controls) for all exposure condition pairs. A dose–response reduction in neurobehavioral performance was hypothesized for SRSs relative to controls, with the most significant reduction occurring in the gasoline with 15% MTBE condition. Baseline POL was subtracted from postexposure POL; the difference between all pairs of exposure conditions of the baseline/postexposure difference was compared (see above equation) between SRSs and controls to assess neurobehavioral effects.

We evaluated two hypotheses for the psychophysiologic variables. First, if subjects had a response to the odor of MTBE, such as that seen for dental odors (*15*), significant changes in psychophysiologic indicators (e.g., heart rate, respiration rate) would be seen shortly after exposure onset when the odor of the exposure was initially perceived. Therefore, for every psychophysiologic variable for each subject, the average baseline

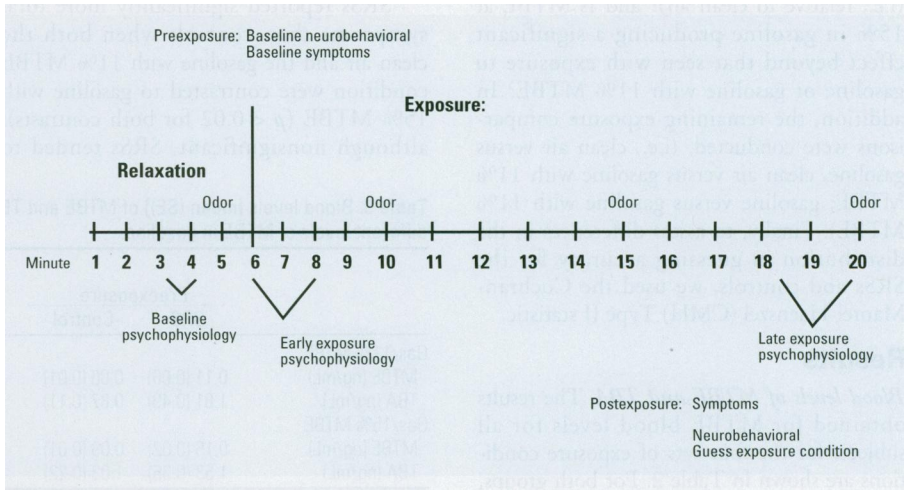

Figure 1. Protocol time line.

values (no-exposure) at 3 and 4 min of the baseline period (1–5 min) were subtracted from the average values measured at 6, 7, and 8 min (early exposure) immediately followed the onset of the exposure period (6–20 min). Second, if MTBE in gasoline had a direct physiologic effect, changes in physiologic measures would be seen later in exposure when MTBE was measurable in blood. Therefore, for each subject, the average of 3 and 4 min was used as a baseline (no-exposure) and was subtracted from min 18, 19, and 20 (late exposure) at the end of the exposure. We chose min 18, 19, and 20 because they would reflect maximum body burden accumulated by a subject after 15 min of exposure. Difference scores between exposure conditions (e.g., gasoline with 15% MTBE minus clean air) were computed from the within-exposure condition difference scores for each subject and were then compared for all exposure condition pairs.

Tests for symptoms, POL, and psychophysiologic responses in min 6, 7, and 8 were one-tailed with a significance level of $p < 0.05$ due to the hypothesis that SRSs relative to controls would show the greatest effects of exposure to gasoline with 15% MTBE. Directionality for baseline versus late exposure psychophysiologic responses was not hypothesized, and therefore, two-tailed tests were conducted for these comparisons.

A series of permutation tests to compare changes in the symptoms, neurobehavioral performance, and psychophysiologic variables were conducted between the exposure conditions clean air versus gasoline with 15% MTBE; gasoline versus gasoline with 15% MTBE; and gasoline with 11% MTBE versus gasoline with 15% MTBE. The questions addressed with these comparisons were; Does gasoline with 15% MTBE produce any significant effect when the maximum possible difference in exposures is compared, (i.e., relative to clean air)? and Is MTBE at 15% in gasoline producing a significant effect beyond that seen with exposure to gasoline or gasoline with 11% MTBE? In addition, the remaining exposure comparisons were conducted, (i.e., clean air versus gasoline, clean air versus gasoline with 11% MTBE; gasoline versus gasoline with 11% MTBE). Finally, to assess differences in the distribution in guessing accuracy for the SRSs and controls, we used the Cochran-Mantel-Haenszel (CMH) Type II statistic.

Results

Blood levels of MTBE and TBA. The results obtained for MTBE blood levels for all subjects for the four sets of exposure conditions are shown in Table 2. For both groups, the preexposure MTBE levels in blood averaged < 0.5 ng/mL. For all subjects (control and SRS), paired *t*-tests were used to contrast difference scores (post- minus preexposure blood levels) of MTBE in blood between all possible pairs of exposure conditions. As shown in Table 3, the gasoline with 15% MTBE and gasoline with 11% MTBE exposure conditions produced significantly greater MTBE blood levels than clean air and gasoline alone. Although TBA was also significantly higher for gasoline with 15% MTBE and gasoline with 11% MTBE exposures relative to clean air and gasoline exposures, no significant difference in TBA was shown between gasoline with 11% MTBE and gasoline with 15% MTBE. When difference scores for MTBE and TBA levels were compared between SRSs and controls for both the gasoline with 15% MTBE and gasoline with 11% MTBE exposure conditions, no significant differences were observed. Thus, SRSs and controls were not different in their uptake or metabolism of MTBE in blood during the period covered by the experiment (Table 3).

Symptom questionnaire. Initially, total symptom scores at baseline in each exposure condition were compared between SRSs and controls (Table 4). Figure 2 reveals that SRSs reported significantly more total symptoms than controls during every exposure condition. Thus, SRSs were more symptomatic than controls regardless of exposure. The primary question of the present study, however, was to determine whether the SRSs responded differently from controls when exposed to gasoline, gasoline with 11% MTBE, or gasoline with 15% MTBE. Therefore, total symptom difference scores (postexposure minus baseline) for each exposure condition were compared to every other exposure condition by subtracting the difference score from one exposure condition from the difference score of the comparison exposure condition (Tables 5 and 6).

SRSs reported significantly more total symptoms than controls when both the clean air and the gasoline with 11% MTBE condition were contrasted to gasoline with 15% MTBE ($p < 0.02$ for both contrasts); although nonsignificant, SRSs tended to report more total symptoms than controls in the gasoline with 15% MTBE exposure relative to the gasoline exposure condition ($p < 0.08$). Total symptom scores were not significantly different between SRSs and controls when air versus gasoline, air versus gasoline with 11% MTBE, and gasoline versus gasoline with 11% MTBE exposures were contrasted (Table 6).

For those total symptom contrasts that were significantly different, secondary permutation analyses were used to contrast symptom subscales. Tables 7 and 8 show all subscale contrasts. For both the clean air versus gasoline with 15% MTBE and gasoline with 11% MTBE versus gasoline with 15% MTBE contrasts, the MTBE, anxiety, and solvent subscales (as defined in Table 1) were significantly higher for SRSs than for controls, but the remainder of symptom subscales were not significantly different between the two groups. Moreover, the environmental quality score for air versus gasoline with 15% MTBE was significantly higher for SRSs than for controls. However, when the Bonferroni correction for multiple comparisons was made (0.05/6; $p < 0.008$), the only subscale contrast that remained significant was that for the MTBE subscale in the gasoline with 11% MTBE versus gasoline with 15% MTBE contrast.

Neurobehavioral measures. For SRSs relative to controls, no significant differences were noted for any of the above contrasts using the composite score of the POL (Tables 9 and 10).

Table 2. MTBE and TBA blood levels for all subjects in each exposure condition [mean (SE)].

	Preexposure (ng/mL)	Postexposure (ng/mL)
MTBE		
Clean air	0.18 (0.04)	0.13 (0.02)
Gasoline	0.23 (0.05)	0.22 (0.06)
Gas/11% MTBE	0.08 (0.02)	1.23 (0.09)
Gas/15% MTBE	0.11 (0.01)	1.75 (0.10)
TBA		
Clean Air	0.80 (0.21)	0.86 (0.23)
Gasoline	0.86 (0.22)	1.04 (0.24)
Gas/11% MTBE	1.20 (0.20)	2.34 (0.20)
Gas/15% MTBE	1.22 (0.19)	2.63 (0.21)

Table 3. Blood levels [mean (SE)] of MTBE and TBA in SRSs versus controls before and after exposure to different levels of MTBE in gasoline.

	Preexposure		Postexposure		Within condition difference[a]		
	SRS	Control	SRS	Control	SRS	Control	*p*
Gas/11% MTBE							
MTBE (ng/mL)	0.11 (0.06)	0.06 (0.01)	1.15 (0.13)	1.28 (0.12)	1.04 (0.12)	1.22 (0.12)	0.35
TBA (ng/mL)	1.81 (0.49)	0.87 (0.11)	2.69 (0.36)	2.14 (0.24)	0.88 (0.41)	1.27 (0.22)	0.37
Gas/15% MTBE							
MTBE (ng/mL)	0.15 (0.02)	0.09 (0.01)	1.76 (0.11)	1.75 (0.14)	1.61 (0.11)	1.66 (0.14)	0.78
TBA (ng/mL)	1.53 (0.35)	1.03 (0.22)	2.95 (0.45)	2.44 (0.21)	1.42 (0.19)	1.41(0.19)	0.98

[a]See "Hypotheses and Statistical Analysis" for calculations.

Psychophysiologic variables. The initial set of permutation analyses compared psychophysiologic changes from baseline (no exposure) to early exposure (min 6, 7, and 8) between all pairs of exposure conditions. No significant differences between SRSs and controls were noted for any of the contrasts of the psychophysiologic variables. The same analyses were repeated comparing changes from baseline (no exposure) to late exposure (min 18, 19, and 20) contrasting all pairs of exposure conditions. No significant differences between exposure conditions were observed for heart rate, respiration rate, finger pulse volume, or finger temperature. However, when clean air was contrasted to gasoline and to gasoline with 15% MTBE, end-tidal CO_2 was significantly higher at postexposure for SRSs relative to controls (not shown). Examination of the end-tidal CO_2 records revealed that some readings were due to incomplete breaths or sniffs during exhalation. When the end-tidal readings associated with sniffs, which do not reflect arterial CO_2, were removed, end-tidal CO_2 was no longer significantly different between SRSs and controls in the clean air versus gasoline 15% MTBE contrast. End-tidal CO_2 remained significantly different between gasoline and clean air. Because this finding was not an effect of MTBE, however, it was not regarded as meaningful.

EMG readings were significantly different between SRSs and controls for all contrasts except clean air versus gasoline with 15% MTBE. However, EMG readings for SRSs increased significantly relative to controls in the gasoline with 11% MTBE, whereas EMG readings increased significantly for controls relative to SRSs in the gasoline with 15% MTBE condition. This pattern of EMG results did not support a consistent effect of MTBE for either group and suggests random fluctuation between exposure conditions and subject groups. In conclusion, SRSs did not show significant psychophysiologic changes in response to the odor of the exposure conditions or in response to biologically detectable levels of MTBE in blood.

Exposure guessing. To assess the effect of subjects' awareness of the exposure conditions, accuracy of subjects' guessing was analyzed. Initially, a kappa statistic was computed to assess overall accuracy [$\kappa = 0.16$; 95% confidence interval (CI), 0.049 – 0.294]. This revealed that subjects were significantly better than chance at guessing the exposure condition. However, examination of performance revealed that subjects' accuracy was only slightly better than chance (chance guessing = 31/124 guesses; 46/124 correct guesses made). To explore whether guessing accuracy was primarily due to the ability to distinguish clean air from the gasoline and MTBE exposure conditions, the kappa statistic calculation was repeated without the clean air condition guess and was not significant ($\kappa = -0.01$; 95% CI, –0.15–0.12). Therefore, it appears that the ability to distinguish between clean air and any other exposure condition was responsible for most of the correct guessing observed. In fact, kappa was significant when guesses made during clean air were compared to guesses for all other exposure conditions ($\kappa = 0.58$; 95% CI, 0.40–0.75). That is, the accuracy rate for clean air was 106 out of 124 guesses. This finding suggests that while subjects generally knew when they were being exposed to chemical mixtures rather than to clean air, they were unable to distinguish gasoline exposure from gasoline with MTBE at 11% (gasoline with low MTBE) or 15% (gasoline with high MTBE).

In addition to computation of the overall guessing accuracy, the possibility that SRSs and controls differed in guessing accuracy was tested. To conduct this test, the number of correct guesses was computed for each subject. The CMH type II statistic revealed no differences in guessing accuracy between the SRSs and controls (mean SRS = 1.66; mean control = 1.37; $p < 0.30$). Therefore, SRSs were no more accurate than controls in correctly identifying the exposure condition. Overall, 79% (19/24 guesses) of SRSs reported exposure to gasoline with MTBE when they were, in fact, exposed to gasoline with either 11% or 15% MTBE. However, their accuracy in discerning whether they were exposed to 11% or 15% MTBE in gasoline was lowest for gasoline with 15% MTBE (42% accuracy for 11% MTBE; 25% accuracy for 15% MTBE). Similar accuracy rates were seen for controls, with a 71% (27/38 guesses) overall accuracy rate for determining that MTBE was present in gasoline. Again, controls were more accurate in guessing that MTBE was present at 11% than at 15% (53% accuracy for 11% MTBE; 21% accuracy for 15% MTBE). Furthermore, 75% (9/12 guesses) of the SRSs and 79% (15/19 guesses) of controls thought they were being exposed to gasoline with MTBE at 11% or 15 % when they were actually exposed to gasoline alone. Table 11 gives all percentages for guessing accuracy.

Odor ratings. To analyze the odor ratings completed within each exposure condition, mixed model analyses of variance were used with group (SRS versus control) as a between-subjects factor and exposure (clean air, gasoline, gasoline with 11% MTBE, gasoline with 15% MTBE) and time (min 5, 10, 15, and 20) as the within-subjects factors. Separate analyses were conducted for each of

Table 4. Permutation analysis: total baseline symptoms before each exposure condition in SRSs versus controls (mean ± SD).

Baseline	SRS	Control	*p*
Clean air	14.3 ± 23.9	1.6 ± 2.7	0.002
Gasoline	10.2 ± 15.7	1.4 ± 2.7	0.0009
Gas/11% MTBE	14.7 ± 23.3	1.3 ± 2.0	0.002
Gas/15% MTBE	18.8 ± 32.8	1.6 ± 2.7	0.0001

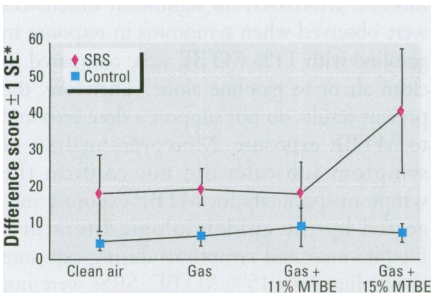

Figure 2. Total symptom response to exposure conditions.

Table 5. Total symptom scores [mean (SE)] at baseline and postexposure for SRSs and controls in each exposure condition.

	Baseline[a]		Postexposure[b]		Within-condition difference[c]	
	SRS	Control	SRS	Control	SRS	Control
Clean air	14.3 (6.9)	1.6 (0.6)	32.0 (17.3)	5.6 (2.2)	17.5 (10.5)	4.0 (1.9)
Gasoline	10.2 (4.5)	1.5 (0.6)	28.5 (7.5)	6.9 (2.7)	18.3 (4.8)	5.4 (2.5)
Gas/11% MTBE	14.7 (6.7)	1.3 (0.5)	32.1 (14.2)	8.8 (5.5)	17.4 (8.9)	7.5 (5.3)
Gas/15% MTBE	18.8 (9.5)	1.6 (0.6)	58.5 (25.7)	7.5 (2.7)	39.7 (17.5)	5.9 (2.6)

[a]Baseline = mins 1–5. [b]Postexposure = immediately following 20-min exposure period. [c]See "Hypotheses and Statistical Analysis" for calculations.

Table 6. Total symptom scores [mean (SE)] at baseline and postexposure for SRSs and controls: permutation analyses of difference scores between exposure conditions.

	SRS	Control	*p*
Gas/15% MTBE – clean air	22.04 (10.23)	1.83 (1.38)	0.02
Gas/15% MTBE – gasoline	21.54 (16.90)	0.46 (1.66)	0.08
Gas/15% MTBE – gas/11% MTBE	22.40 (10.97)	–1.69 (4.13)	0.02
Gasoline – clean air	0.50 (10.83)	1.37 (2.04)	0.60
Gas/11% MTBE – clean air	–0.36 (6.12)	3.53 (4.63)	0.66
Gas/11% MTBE – gasoline	–0.86 (10.18)	2.15 (2.98)	0.63

the following odor ratings completed at each point in time: irritation, intensity, and pleasantness. Table 12 shows the results of these analyses. The group effect was significant for irritation and intensity and approached significance for pleasantness, with the SRSs reporting more irritation, intensity, and less pleasantness than controls over all exposures and time points. A significant main effect of exposure was seen for intensity and irritation ratings but not for pleasantness, and a significant time effect was observed for all three ratings. Because the exposure × time interaction was significant for intensity and pleasantness, this interaction was graphed and interpreted. For all three qualities, ratings changed less over time for clean air exposure than for any of the other exposures. Differences in ratings of intensity and pleasantness between time 1 (ratings before exposure) and the remainder of the ratings during the gasoline, gasoline with 11% MTBE, and gasoline with 15% MTBE were largely responsible for the significant exposure × time interaction for both groups. Although the interaction (group × exposure × time) was nonsignificant, the SRSs showed a steady increase in irritation over time for the gasoline with 15% MTBE condition, which was not as evident for the gasoline or gasoline with 11% MTBE exposure conditions or for the controls. The same was not true for intensity ratings, which appeared to increase when exposure began and remained at approximately the same level for the remainder of the exposure period for both groups. Although the exposure × time × group interaction was not significant for pleasantness ($p < 0.06$), pleasantness changed less over time in all exposure conditions for controls relative to SRSs.

Table 7. Subscale symptom scores [mean (SE)] at baseline and postexposure for SRSs and controls in each exposure condition.

	Baseline[a]		Postexposure[b]		Within-condition difference[c]	
	SRS	Control	SRS	Control	SRS	Control
MTBE						
Clean air	4.6 (1.9)	0.6 (0.3)	10.1 (4.8)	2.3 (0.8)	5.5 (3.0)	1.7 (0.7)
Gasoline	2.8 (0.7)	0.6 (0.2)	10.7 (2.4)	2.8 (1.2)	7.9 (2.0)	2.2 (1.2)
Gas/11% MTBE	3.8 (1.6)	0.5 (0.2)	9.2 (2.5)	3.5 (1.6)	5.4 (1.8)	2.9 (6.2)
Gas/15% MTBE	5.3 (2.3)	0.5 (0.2)	18.5 (6.0)	2.7 (0.8)	13.2 (4.7)	2.2 (0.8)
Anxiety						
Clean air	1.4 (0.7)	0.2 (0.1)	4.5 (2.7)	1.1 (0.7)	3.1 (2.1)	1.0 (0.7)
Gasoline	0.3 (0.3)	0.0 (0.0)	5.3 (1.9)	0.6 (0.3)	5.0 (1.7)	0.6 (0.3)
Gas/11% MTBE	1.3 (0.5)	0.1 (0.1)	3.9 (2.3)	1.2 (1.0)	2.6 (2.2)	1.1 (1.0)
Gas/15% MTBE	2.8 (1.9)	0.6 (0.4)	10.8 (5.9)	1.3 (0.6)	8.1 (4.3)	0.7 (0.6)
Depression						
Clean air	1.8 (0.6)	0.4 (0.2)	6.1 (3.3)	0.4 (0.3)	4.3 (3.3)	−1.2 (0.3)
Gasoline	5.0 (3.7)	0.4 (0.4)	4.0 (1.5)	1.4 (0.6)	−1.0 (2.7)	1.0 (0.5)
Gas/11% MTBE	4.2 (2.0)	0.0 (0.0)	7.8 (3.3)	1.1 (0.8)	3.6 (2.1)	1.1 (0.8)
Gas/15% MTBE	5.0 (2.7)	0.1 (0.1)	13.3 (5.7)	0.9 (0.5)	8.3 (4.3)	0.8 (0.5)
Breathing						
Clean air	3.5 (3.3)	0.0 (0.0)	4.8 (3.7)	0.5 (0.4)	1.3 (0.6)	0.5 (0.4)
Gasoline	0.1 (0.1)	0.0 (0.0)	2.6 (1.7)	0.2 (0.2)	2.5 (1.7)	0.2 (0.2)
Gas/11% MTBE	2.0 (1.6)	0.1 (0.1)	4.4 (4.4)	1.6 (1.4)	2.4 (2.9)	1.5 (1.4)
Gas/15% MTBE	1.5 (1.5)	0.1 (0.1)	5.2 (4.8)	1.1 (0.9)	3.8 (3.3)	1.0 (0.9)
Solvent						
Clean air	2.9 (1.3)	0.4 (0.2)	6.5 (3.2)	1.3 (0.4)	3.6 (1.8)	0.9 (0.3)
Gasoline	2.1 (0.7)	0.4 (0.1)	5.9 (1.4)	1.7 (0.6)	3.8 (0.9)	1.4 (0.6)
Gas/11% MTBE	3.5 (1.7)	0.5 (0.2)	6.8 (2.8)	1.5 (0.8)	3.3 (1.5)	1.0 (0.7)
Gas/15% MTBE	4.2 (2.4)	0.4 (0.2)	10.7 (4.7)	1.6 (0.5)	6.5 (2.6)	1.2 (0.4)
Environment						
Clean air	2.8 (0.2)	2.8 (0.1)	2.8 (1.9)	2.9 (0.1)	0.0 (0.1)	0.1 (0.0)
Gasoline	2.7 (0.1)	2.9 (0.1)	3.3 (0.2)	3.0 (0.1)	0.6 (0.1)	0.1 (0.1)
Gas/11% MTBE	2.6 (0.1)	2.8 (0.1)	3.2 (0.1)	3.1 (0.1)	0.6 (0.1)	0.3 (0.1)
Gas/15% MTBE	2.6 (0.1)	2.8 (0.1)	3.2 (0.2)	3.1 (0.1)	0.6 (0.1)	0.3 (0.0)

[a]Baseline = min 1–5. [b]Postexposure = immediately following 20 min exposure period. [c]See "Hypotheses and Statistical Analysis" for calculations.

Table 8. Subscale symptom scores [mean (SE)] at baseline and post-exposure for SRSs and controls: permutation analysis of difference scores between exposure conditions.[a]

	SRS	Control	p
MTBE			
Gas/15% MTBE – clean air	7.64 (4.06)	0.53 (0.64)	0.02
Gas/15% MTBE – gas/11% MTBE	7.74 (3.96)	−0.75 (1.40)	0.001[a]
Anxiety			
Gas/15% MTBE – clean air	4.97 (2.56)	−0.26 (0.34)	0.009
Gas/15% MTBE – gas/11% MTBE	5.42 (2.67)	−0.39 (0.89)	0.02
Depression			
Gas/15% MTBE – clean air	4.03 (2.79)	0.79 (0.58)	0.11
Gas/15% MTBE – gas/11% MTBE	4.72 (3.40)	−0.26 (0.88)	0.08
Breathing			
Gas/15% MTBE – clean air	2.50 (3.14)	0.46 (0.53)	0.34
Gas/15% MTBE – gas/11% MTBE	1.35 (0.98)	−0.53 (0.54)	0.08
Solvent			
Gas/15% MTBE – clean air	2.90 (0.16)	0.32 (0.29)	0.03
Gas/15% MTBE – gas/11% MTBE	3.16 (1.63)	0.24 (0.59)	0.05
Environment			
Gas/15% MTBE – clean air	0.53 (0.16)	0.25 (0.06)	0.04
Gas/15% MTBE – gas/11% MTBE	−0.04 (0.08)	0.01 (0.10)	0.77

[a]Bonferroni correction (0.05/6; $p < 0.008$).

Discussion

Exposure to gasoline with 15% MTBE resulted in significantly more symptoms among SRSs relative to controls than did exposure to clean air or to gasoline with 11% MTBE. In addition, SRSs reported significantly more symptoms than controls during the baseline period preceding all exposure conditions, suggesting heightened sensitivity, regardless of exposure, among this group. It is interesting to note that TBA levels were somewhat higher for the SRSs than for controls before exposure to gasoline with 11% MTBE and gasoline with 15% MTBE (Table 3) but not before exposure to gasoline or to clean air (not shown). This suggests that before at least two of the exposure conditions, SRSs were exposed at some time during the previous week to MTBE. However, the differences in TBA between SRSs and controls were not significant and were not significantly associated with baseline symptom scores. Therefore, the preexposure difference in TBA probably does not account for baseline differences in symptoms. Although SRSs also reported more symptoms than controls when exposed to gasoline with 15% MTBE relative to gasoline alone, this difference was not statistically significant. Moreover, no significant differences were observed when symptoms in response to gasoline with 11% MTBE were compared to clean air or to gasoline alone. Therefore, the present results do not support a dose response to MTBE exposure. Moreover, analysis of symptom subscales did not confirm the symptom specificity for MTBE exposure suggested by the epidemiologic literature. Despite increased symptoms during exposure to gasoline with 15% MTBE, SRSs were not impaired relative to controls in overall performance of a driving simulation task, nor did

they show significant psychophysiologic responses early or late in the exposure period. Thus, controlled exposure to gasoline with 15% MTBE, while producing more symptoms, did not cause impaired performance or psychophysiologic changes.

The present study also supports the reodorant study (*27*) finding that the majority of subjects were unable to discriminate exposure to gasoline from exposure to gasoline with MTBE. Thus, subjects were indeed blind to the specific exposure conditions, and changes in symptoms could not be attributed to either response bias due to awareness of the exposure condition and/or anxiety in response to odor. First, neither SRSs nor controls were accurate in identifying specific exposure conditions. Subjects were most accurate in distinguishing clean air from chemical exposures. However, neither SRSs nor controls were able to distinguish whether MTBE was present at 11% or 15%; and a majority of both groups thought MTBE was present when they were exposed to gasoline only. Furthermore, if knowledge of MTBE exposure was the determinant of symptoms, then one would not expect the increased symptom reports observed from 11% MTBE to 15% MTBE. In the present study, symptom responses could not be explained entirely by awareness of MTBE exposure.

A negative emotional association with the odor of MTBE, particularly among the SRSs, could produce increased heart rate and muscle tension or decreased end-tidal CO_2. Such physiologic responses are associated with symptoms such as headache and dizziness, the same symptoms as those attributed to MTBE exposure. However, neither SRSs nor controls exhibited significant changes on any psychophysiologic measure in response to odor cues perceived in the initial phase of exposure to gasoline with 15% MTBE. Thus, psychophysiologic indicators did not support that the odor of MTBE produced a generalized sympathetic arousal as would accompany a panic state at least for this relatively small subgroup of SRSs.

The periodic ratings of odor intensity, irritation, and pleasantness during each exposure also did not suggest that the response of either the SRSs or controls to the odors changed differentially (i.e., exposure × time × group interaction). Although the magnitude of the SRS response to the odors of all exposure conditions, including clean air, was greater than the controls, the response pattern over time was not different between the groups. Both groups rated the odor of exposure to the gasoline with or without MTBE as increasingly more intense and less pleasant over time with the greatest increases occurring between time 1 (5 min), before exposure and the onset of exposure to gasoline with or without MTBE. This change in ratings over time, however, did not occur for the clean air condition. Although not significant, the pattern of rating for pleasantness suggested that relative to the controls, the SRSs rated the odor as increasingly unpleasant over the time they were exposed to all gasoline mixtures.

Table 9. Composite score [mean (SE)] Performance on Line for SRSs and controls in each exposure condition.

	Baseline		Postexposure		Within-condition difference[a]	
	SRS	Control	SRS	Control	SRS	Control
Clean air	92.9 (10.4)	101.7 (4.5)	99.6 (7.1)	104.4 (4.6)	6.7 (12.1)	2.7 (1.3)
Gasoline	88.0 (8.4)	103.2 (5.1)	88.4 (8.6)	104.9 (4.5)	0.5 (2.1)	1.8 (1.6)
Gas/11% MTBE	94.9 (8.1)	101.2 (4.7)	94.4 (7.0)	103.9 (4.5)	−0.5 (2.5)	2.7 (1.9)
Gas/15% MTBE	84.7 (9.5)	101.0 (4.3)	79.4 (11.7)	102.5 (4.0)	−5.3 (5.6)	1.5 (1.1)

[a]See "Hypotheses and Statistical Analysis" for calculations.

Table 10. Composite score [mean (SE)] Performance on Line for SRSs and controls: permutation analyses of difference scores between exposure conditions.

	SRS	Control	*p*
Gas/15% MTBE – clean air	−12.0 (12.7)	−1.2 (1.5)	0.30
Gas/15% MTBE – gasoline	5.8 (4.2)	0.3 (1.7)	0.17
Gas/15% MTBE – gas/11% MTBE	4.9 (5.1)	1.2 (2.3)	0.49
Gasoline – clean air	−6.3 (12.1)	−0.9 (2.1)	0.69
Gas/11% MTBE – clean air	−7.2 (11.9)	0.0 (2.3)	0.56
Gas/11% MTBE – gasoline	0.9 (2.2)	−1.0 (2.7)	0.63

Table 11. Subjects' awareness of exposure: percentage of subjects' guesses by exposure condition.

	Subject's guess			
Exposure condition	Clean air (%)	Gasoline (%)	MTBE 11% (%)	MTBE 15% (%)
SRS (*n* = 12)				
Clean air	75 (9)	8 (1)	8 (1)	8 (1)
Gasoline	0 (0)	25 (3)	67 (8)	8 (1)
Gas/11% MTBE	0 (0)	33 (4)	42 (5)	25 (3)
Gas/15% MTBE	0 (0)	8 (1)	67 (8)	25 (3)
Total guesses	19 (9/48)	19 (9/48)	46 (22/48)	17 (8/48)
Controls (*n* = 19)				
Clean air	47 (9)	16 (3)	32 (6)	5 (1)
Gasoline	5 (1)	16 (3)	63 (12)	16 (3)
Gas/11% MTBE	16 (3)	16 (3)	53 (10)	16 (3)
Gas/15% MTBE	5 (1)	21 (4)	53 (10)	21 (4)
Total guesses	18 (14/76)	17 (13/76)	50 (38/76)	14 (11/76)

The value in parentheses = *n*.

Table 12. Odor ratings: mixed model analysis of variance for the effects of group, exposure condition, and time.

	Intensity				Irritation				Pleasantness			
	Type III SS	*df*	*F*	*p*	Type III SS	*df*	*F*	*p*	Type III SS	*df*	*F*	*p*
Main effects												
Group[a]	13511.9	1,29	4.73	0.04[d]	13265.2	1,29	5.77	0.03[d]	66.0	1,26	4.07	0.05
Exposure[b]	14689.8	3,87	15.02	0.0001[e]	6285.5	3,87	10.05	0.0001[e]	21.0	3,78	2.05	0.12
Time[c]	22246.6	3,87	34.89	0.0001[f]	6701.7	3,87	14.27	0.0001[f]	116.7	3,78	24.2	0.0001[f]
Interaction effects												
Exposure × group	608.4	3,87	0.62	0.55	1633.3	3,87	2.61	0.07	2.9	3,78	0.3	0.82
Time × group	1239.3	3,87	1.94	0.16	409.1	3,87	0.87	0.41	13.7	3,78	2.8	0.07
Exposure × time	6474.3	9,261	8.61	0.0001	1694.4	9,261	2.42	0.09	32.6	9,234	5.4	0.0001
Exposure × time × group	762.2	9,261	1.01	0.41	821.6	9,261	1.18	0.32	12.0	9,234	2.0	0.06

SS, sum of squares.
[a]SRSs versus controls. [b]Clean air, gasoline, gas/11% MTBE, or gas/15% MTBE. [c]Min 5, 10, 15, or 20. [d]SRS > controls. [e]Gasoline, gas/11% MTBE, gas/15% MTBE > clean air. [f]Min 10, 15, 20 > 5.

The changing pattern of ratings over time and between exposure conditions was not significant for ratings of irritation. Odor ratings during exposure, however, do not support a significant, differential odor response to gasoline, gasoline with 11% MTBE, or gasoline with 15% MTBE for either group. Thus, symptom results do not appear to be mediated by a negative or specific hedonic response to MTBE at 15% in gasoline.

The current results reveal that the SRSs chosen were indeed "sensitive" as illustrated by greater baseline symptom scores and odor ratings of irritation and intensity than controls during every experimental condition. It is noteworthy that, in contrast to the present findings, no previous controlled exposure study has documented significantly increased symptoms in response to MTBE exposure (*7–9*). However, this is the first study to test directly the responses of individuals not only self-reported as sensitive to MTBE but also apparently more generally sensitive as indicated by increased symptoms regardless of exposure. Other investigators have recommended this approach, as in the case of sick building syndrome, yet it is seldom undertaken (*29*). Previous studies, in which sick building syndrome (SBS) subjects were exposed to mixtures of volatile organic compounds known to pollute indoor air, also found significantly increased symptom reports among the SBS subjects relative to controls (*30,31*). Thus, direct testing of subgroups reporting unexpected symptoms in response to low-level exposures may be necessary to understand reported health effects. The present study, however, also highlights problems with the feasibility of studies with sensitive subgroups given the constraints required for controlled exposures, the relatively small number of affected individuals in the population, and the understandable reticence of affected individuals to undergo exposure to the chemical of concern.

The second factor that differentiated this study from previous controlled exposure studies with MTBE was that exposure conditions were designed to approximate what is experienced in daily life. Therefore, subjects were exposed to MTBE in gasoline at concentrations comparable to those encountered at a gasoline station (*16*). All previous studies involved exposure to pure MTBE. As suggested by Nihlen et al. (*7*), it may be that when MTBE is mixed with gasoline, a different effect occurs from that seen with pure MTBE.

The intent of the present research approach was to test directly both the exposure conditions and the individuals presumed to be at highest risk (i.e., SRSs). While ecologically valid, this approach placed significant restrictions on subject recruitment and therefore, on generalizability of results. The number of SRS subjects who were healthy and also willing to participate in an exposure study was small relative to the numbers cited by community groups as adversely affected by MTBE. Therefore, the present results cannot be generalized to the entire population of SRS individuals nor to those who are SRSs with other health conditions. Those most sensitive to MTBE may have been least likely to volunteer for an exposure study. This self-selection bias could partially explain the present findings. Furthermore, the relatively small sample size may have reduced the power of the study. For example, the SRSs reported more symptoms at 15% MTBE versus clean air and 11% MTBE, but the contrast with gasoline failed to reach statistical significance. This variability in results may be due to the small sample size, but increasing sample size may or may not increase the ability to detect significant effects for any of the variables measured.

Although a dose–response effect was not observed, the results suggested that increased symptoms were associated with a threshold. This possibility is plausible because complaints in New Jersey occurred primarily during oxygenated fuel season when MTBE was present at 15%, but not during the reformulated gas season when 11% MTBE was present in gasoline. Unfortunately, quantitative values for each participant's exposure to MTBE before the study were not available. Although baseline TBA levels and questionnaires on vehicle use suggest that some SRSs may have had more exposure to MTBE prior to their participation in the study, the effect on the threshold observed cannot be assessed from the data.

Time constrained the use of additional neurobehavioral tests or other objective markers (e.g., tear film) that may have been more sensitive to the effects of exposure. For example, the SRSs reported more symptoms when exposed to MTBE at 15% in gasoline, yet neurobehavioral performance, as measured, was not compromised as a result of the exposure. Thus, any impact of these symptoms on daily behavioral functions connected to driving was not shown. With different test batteries, previous controlled exposure studies also reported no significant neurobehavioral performance decrements (*8*), lending validity to the negative neurobehavioral results found here.

Finally, it is also possible that use of longer exposure durations or exposure conditions that reflect ongoing exposure while driving may have shown greater effects on performance. For example, anecdotal reports indicate that SRS subjects reported symptom exacerbation while driving in traffic where exhaust emissions are prevalent. The latter could not have been completed in a CEF study because of the high levels of carbon monoxide in the exhaust and the complex composition of exhaust in the atmosphere, (e.g., fresh versus aged exhaust that includes particles and gases).

Conclusions

Compared to controls, individuals who reported they were sensitive to MTBE reported more symptoms at baseline independent of exposure condition, suggesting a generic, nonspecific sensitivity. Moreover, these SRSs reported more symptoms than controls in response to gasoline with 15% MTBE than to clean air or to gasoline with 11% MTBE. This differential response to gasoline with 15% MTBE was not explained by specific knowledge of exposure or reactions to the odor of exposure. Despite increased symptoms in response to gasoline with 15% MTBE, SRSs did not show any concomitant decrements in simulated driving performance tests, and did not manifest any significant psychophysiologic responses with exposure. Furthermore, the present study did not support a dose response to MTBE exposure nor the specific symptoms associated with MTBE that were suggested in previous epidemiologic studies.

References and Notes

1. Clean Air Act Amendments of 1990. Public Law. 101-549, 1990.
2. Beller M, Schloss M, Middaugh J. Evaluation of Health Effects from Exposure to Oxygenated Fuel in Fairbanks, Alaska (Middaugh J, ed). Bulletin 26. Anchorage, AK:Department of Health and Social Services, 1992.
3. Chandler B, Middaugh JP. Potential Illness due to Exposure to Oxygenated Fuel (Middaugh J, ed). Bulletin 1. Anchorage, AK:Department of Health and Social Services, 1993.
4. Moolenaar RL, Hefflin BJ, Ashley DL, Middaugh JP, Etzel RA. Methyl tertiary butyl ether in human blood after exposure to oxygenated fuel in Fairbanks, Alaska. Arch Environ Health 49:402–409 (1994).
5. White MC, Johnson CA, Ashley DL, Buchta T, Pelletier DJ. Exposure to methyl tertiary-butyl ether from oxygenated gasoline in Stamford, Connecticut. Arch Environ Health 50:183–189 (1995).
6. HEI. The Potential Health Effects of Oxygenates Added to Gasoline. A Review of the Current Literature. Topsfield, MA:Health Effects Institute, 1996.
7. Nihlen A, Walinder R, Lof A, Johanson G. Experimental exposure to methyl tertiary-butyl ether. II. Acute effects in humans. Toxicol Appl Pharmacol 148:281–287 (1998).
8. Prah JD, Goldstein GM, Devlin R, Otto S, Ashley D, House D, Cohen KL, Gerrity T. Sensory, symptomatic, inflammatory, and ocular responses to and the metabolism of methyl tertiary butyl ether in a controlled human exposure experiment. Inhal Toxicol 6:521–538 (1994).
9. Cain WS, Leaderer BP, Ginsberg GL, Andrews LS, Cometto-Muniz JE, Gent JF, Buck M, Berglund LG, Mohsenin V, Monahan E, Kjaergaard S. Acute exposure to low-level methyl tertiary-butyl ether (MTBE): human reactions and pharmacokinetic responses. Inhal Toxicol 8:21–48 (1996).
10. Fiedler N, Mohr SN, Kelly-McNeil K, Kipen HM. Response of sensitive groups to MTBE. Inhal Toxicol 6:539–552 (1994).
11. Lorig TS. Human EEG and odor response. Prog Neurobiol 33:387–398 (1989).
12. Ludvigson HW, Rottman TR. Effects of ambient odors of lavender and cloves on cognition, memory, affect and mood. Chem Senses 14(4):525–536 (1989).

13. Alaoui-Ismaili O, Vernet-Maury E, Dittmar A, Delhomme G, Chanel J. Odor hedonics: connection with emotional response estimated by autonomic parameters. Chem Senses 22:237–248 (1997).
14. Alaoui-Ismaili O, Robin O, Rada H, Dittmar A, Vernet-Maury E. Basic emotions evoked by odorants: comparison between autonomic responses and self-evaluation. Physiol Behav 62(4):713–720 (1997).
15. Robin O, Alaoui-Ismaili O, Dittmar A, Vernet-Maury A. Emotional responses evoked by dental odors: an evaluation from autonomic parameters. J Dent Res 77(8): 1638–1646 (1998).
16. Lioy PJ, Weisel CP, Jo W-K, Pellizzari E, Raymer JH. Microenvironmental and personal measurements of methyl-tertiary butyl ether (MTBE) associated with automobile use activities. J Expos Anal Environ Epidemiol 4:427–447 (1994).
17. Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA. Manual for the State-Trait Anxiety Inventory (Form Y). Palo Alto, CA:Consulting Psychologists Press, 1983.
18. Beck AT. Beck Depression Inventory. San Antonio, TX:Harcourt, Brace Jovanovich, Inc, 1978.
19. Green HG, Dalton P, Cowart B, Shaffer G, Rankin K, Higgins J. Evaluating the labeled magnitude scale for measuring sensations of taste and smell. Chem Senses 21:323–334 (1996).
20. Mohr S, Fiedler N, Weisel C, Kelly-McNeil K. Health effects of MTBE among New Jersey garage workers. Inhal Toxicol 6:553–562 (1994).
21. Hudnell HK, Otto DA, House DE, Molhave L. Exposure of humans to a volatile organic mixture. II. Sensory. Arch Environ Health 47:31–38 (1992).
22. Dalton P, Wysocki CJ, Brody MJ, Lawley HJ. Perceived odor, irritation, and health symptoms following short-term exposure to acetone. Am J Ind Med 31:558–569 (1997).
23. Mills KC, Parkman KM, Spruill SE. A PC-based software test for measuring alcohol and drug effects in human subjects. Alcohol Clin Exp Res 20:1582–1591 (1996).
24. Hassett J. A Primer of Psychophysiology. New York:WH Freeman, 1978.
25. Hoffmann U, Essfeld D, Stegemann J. Comparison of arterial, end-tidal and transcutaneous PCO_2 during moderate exercise and external CO_2 during moderate exercise and external CO_2 loading in humans. Eur J Appl Physiol 61:1–4 (1990).
26. Kavanagh BP, Sandler AN, Turner KE, Wick V, Lawson S. Use of end-tidal PCO_2 as noninvasive measurement of arterial PCO_2 in extubated patients recovering from general anaesthesia. J Clin Monit 8:226–230 (1992).
27. Opiekun RE, Kelly-McNeil K, Knasko S, Lioy P, Fiedler N. A controlled short-term exposure study to investigate the odor differences among three different formulations of gasoline. Chem Senses (in press).
28. Fisher RA. The Design of Experiments. New York:Hafner Press, 1971.
29. Prah JD, Case MW, Goldstein GM. 1998 Equivalence of sensory responses to single and mixed volatile organic compounds at equimolar concentrations. Environ Health Perspect 106:739–744 (1998).
30. Molhave L, Back B, Pedersen OF. Human reactions to low level concentrations of volatile organic compounds. Environ Int 12:167–175 (1986).
31. Kjaergaard SK, Molhave L, Pedersen OF. Human reactions to a mixture of indoor air volatile organic compounds. Atmos Environ 25:1417–1426 (1991).